CLAIMS

1. A composition (**K**) for immobilization of biological macromolecules in hydrogels by using a copolymerization which have the following formula:

$$K = aA + bB + cC + dD + eE$$

wherein:

A is a monomer based on derivatives of acrylic and methacrylic acids;

B is a water soluble cross-linking agent;

C is a biological modified macromolecule bearing an unsaturated group;

D is a water soluble compound as a medium component for performing a copolymerization;

E is water, and

a, b, c, d, e are percentages (X) of each ingredient in the composition (for solids X = m/v×100% and for liquids X = v/v×100%) having the total content of monomer and cross-linking agent ranging from 3 to 40% ($3 \leq (a+b) \leq 40\%$), and a monomer to cross-linking agent ratio being within a range of 97:3 to 60:40 and percentages of **C, D,** and **E** ingredients being within a range of $0.0001\% \leq c \leq 10\%$; $0\% \leq d \leq 90\%$; $5\% \leq e \leq 95\%$; is useful for production of biochips.

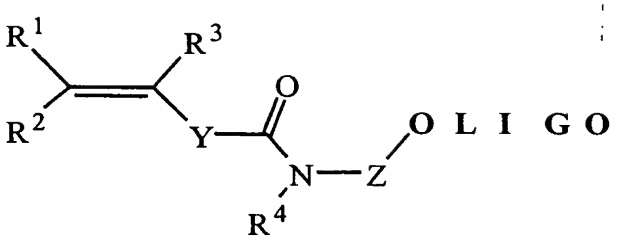
2. The composition of claim 1 comprising acrylamide, methacrylamide, *N*-[tris(hydroxymethyl)methyl]acrylamide, and 2-hydroxyethylmethacrylate as monomer (**A**).

3. The composition of claim 1 wherein monomers being used separately or as a mixture.

4. The composition of claim 1 comprising *N,N'*-methylenbisacrylamide, *N,N'*-ethylenbismethacrylamide, *N,N'*-(1,2-dihydroxyethylene)bisacrylamide, and polyethylene glycol diacrylate as cross-linking agent (**B**).

5. The composition of claim 1 wherein cross-linking agents being used separately or as a mixture.

6. The composition of claim 1 wherein use is made of an oligonucleotide of general formula (I):



(I)

wherein

OLIGO represents an oligonucleotide;

R^1, R^2, R^3 represent H, alkyl C_1-C_6, Ph, $PhCH_2$- ;

Z is $(CH_2)_nCH(CH_2OH)CH_2OX$ where n = 1-6; or $(CH_2)_n$-OX where n = 2-6;

X is a phosphodiester group binding an unsaturated moiety to 5'- and/or 3'-end of the oligonucleotide;

R^4 represents H, $(CH_2)_nOH$ where n = 2-6;

Y is $(p\text{-}C_6H_4)_n$ where n = 0-2,

as a modified biological macromolecule (**C**) being immobilized.

7. The composition of claim 1 wherein use is made of the DNA of general formula **(II)**

5'–X–O–DNA–3'–O–Z (II)

wherein

DNA represents a DNA fragment,

X is H or H_2PO_3, Z represents –CO–Y–CR^1=CR^2R^3

or

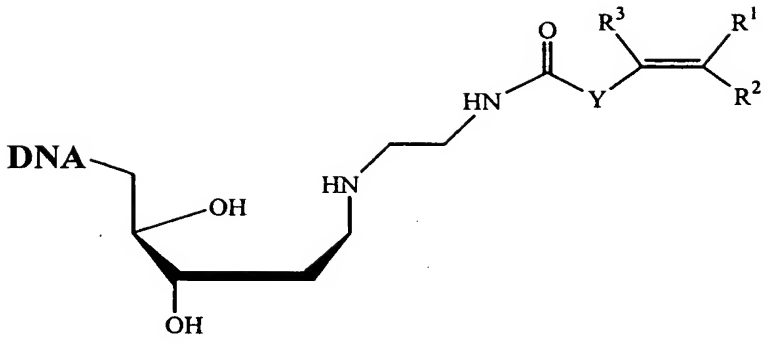
X is –CO–Y–CR^1=CR^2R^3, Z is H or H_2PO_3,

R^1, R^2, R^3 are H, alkyl C_1-C_6, Ph, $PhCH_2$– ;

Y represents $(p\text{-}C_6H_4)_n$ where n = 0-2

as a modified biological macromolecule (**C**) being immobilized.

8. The composition of claim 1 wherein use is made of the DNA of general formula **(III)**:



(III)

wherein:

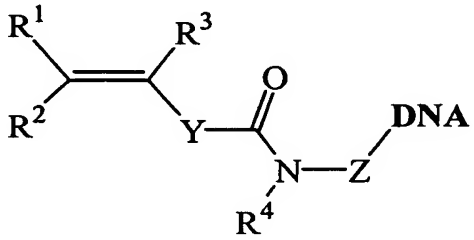
DNA represents a DNA fragment;

R^1, R^2, R^3 are H, alkyl C_1-C_6, Ph, $PhCH_2$– ;

Y is $(p\text{-}C_6H_4)_n$ where n = 0-2,

as a modified biological macromolecule (**C**) being immobilized.

9. The composition of claim 1 wherein use is made of the DNA of general formula **(IV)**



IV

wherein:

DNA represents a DNA fragment;

R^1, R^2, R^3 are H, alkyl C_1-C_6, Ph, $PhCH_2$– ;

Y is $(p\text{-}C_6H_4)_n$ where n = 0-2;

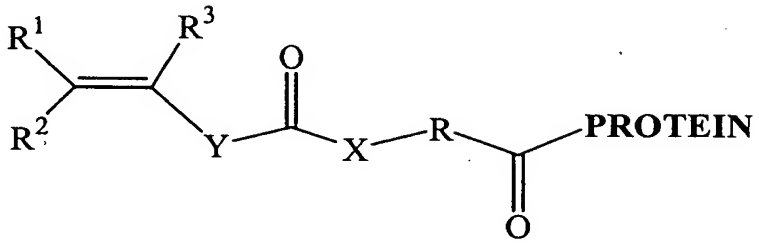
R^4 represents H, $(CH_2)_nOH$ where n = 2-6;

Z is $(CH_2)_nCH(CH_2OH)CH_2OX$ where n = 1-6; or $-(CH_2)_n-OX$ where n = 2-6;

X is a phosphodiester group binding an unsaturated moiety to 5'- and/or 3'-end of the DNA fragment,

as a modified biological macromolecule (**C**) being immobilized.

10. The composition of claim 1 wherein use is made of the protein of general formula **(V)**



V

wherein

R^1, R^2, R^3 are H, alkyl C_1-C_6, Ph, $PhCH_2$–;

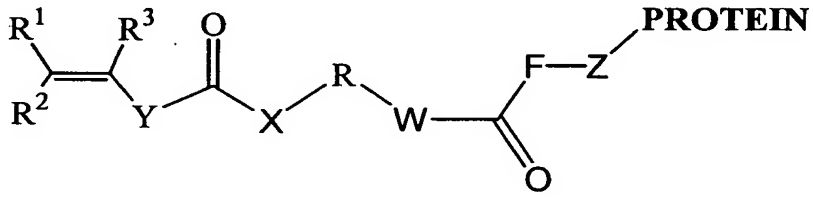
X is NH, O, CH_2, S;

Y represents $(p\text{-}C_6H_4)_n$ where n = 0-2;

R is $(CH_2)_n$, $(CH_2CH_2O)_n$, n = 1- 20,

as a modified biological macromolecule (C) being immobilized.

11. The composition of claim 1 wherein use is made of the protein of general formula **(VI)**



VI

wherein

R^1, R^2, R^3 are H, alkyl C_1-C_6, Ph, $PhCH_2$- ;

X is NH, O, S, CH_2 ;

Y is $(p\text{-}C_6H_4)_n$, where n = 0-2;

R is $(CH_2)_n$, $(CH_2CH_2O)_n$, n = 1-20;

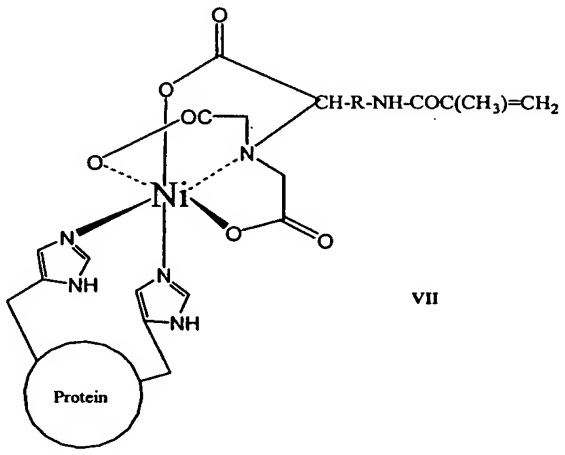
W is NH, O, CH_2;

F is $(CH_2)_n$, n=1, 2;

Z =NH, S

as a modified biological macromolecule (**C**) being immobilized.

12. The composition of claim 1 wherein use is made of the protein of general formula (**VII**)



VII

wherein R represents $(CH_2)_n$, $(CH_2CH_2O)_n$, n = 1–20;
as a modified biological macromolecule (**C**) being immobilized.

13. The composition of claim 1 wherein use is made of a water soluble high-boiling organic compound as a component (**D**) of medium for performing a copolymerization.

14. The composition of claim 13 wherein use is made of *N,N*-dimethylformamide and dimethylsulfoxide as a water soluble high-boiling organic compound.

15. The composition of claim 1, characterizing in that use is made of a water soluble polyhydric compound as a component of medium for performing the photo initiated polymerization.

16. The composition of claim 15, characterizing in that glycerol, sucrose or polyvinyl alcohol are used as water soluble polyhydric compounds.

17. A method for preparing a composition (**K**) according to claim 1 for immobilization of biological macromolecules in hydrogels by using a copolymerization consisting in that components of mixture of the following formula:

$$K = aA + bB + cC + dD + eE$$

wherein:

A is a monomer based on derivatives of acrylic and methacrylic acids;

B is a water soluble cross-linking agent;

C is a biological modified macromolecule bearing an unsaturated group;

D is a water soluble compound as a medium component for performing a copolymerization;

E is water, and

a, b, c, d, e are percentages (X) of any ingredient in the composition (for solids X = m/v×100% and for liquids X = v/v×100%) having the total content of monomer and cross-linking agent ranging from 3 to 40% ($3 \leq$ **(a+b)** $\leq 40\%$), and a monomer to cross-linking agent ratio being within a range of 97:3 to 60:40 and percentages of **C, D**, and **E** ingredients being within a range of $0.0001\% \leq$ **c** $\leq 10\%$; $0\% \leq$ **d** $\leq 90\%$; $5\% \leq$ **e** $\leq 95\%$;

are mixed until the formation of a homogeneous solution which being degassed and being useful for production of biochips.

18. Modified DNA fragments of the following structure:

5'–X–O–DNA–3'–O–Z **(II)**

wherein

DNA represents a DNA fragment,

X is H or H_2PO_3, Z represents $-CO-Y-CR^1=CR^2R^3$

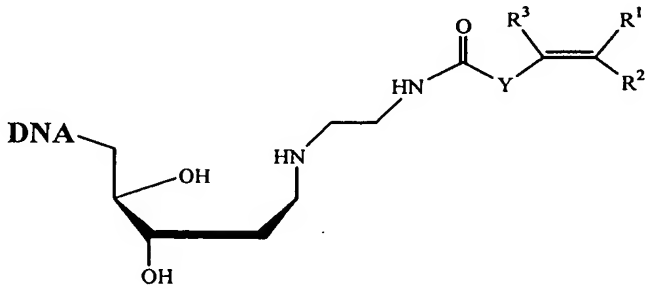
or

X is $-CO-Y-CR^1=CR^2R^3$, Z is H or H_2PO_3,

Y represents $(p\text{-}C_6H_4)_n$ where n = 0-2;

being prepared by direct acylation of DNA fragments with anhydrides of unsaturated acids;

or formula **(III)**:



(III)

wherein:

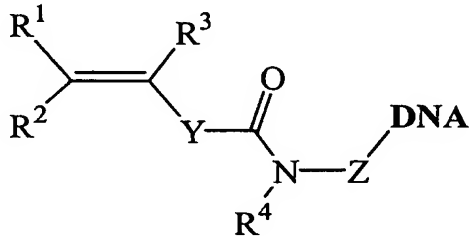
DNA represents a DNA fragment;

R^1, R^2, R^3 are H, alkyl C_1-C_6, Ph, $PhCH_2$– ;

Y is $(p\text{-}C_6H_4)_n$ where n = 0-2,

being prepared by reductive amination of the purine free DNA followed by acylation of amine derivative with activated esters of unsaturated acids;

or



IV

wherein:

DNA represents a DNA fragment;

R^1, R^2, R^3 are H, alkyl C_1-C_6, Ph, $PhCH_2$– ;

Y is $(p\text{-}C_6H_4)_n$ where n = 0-2;

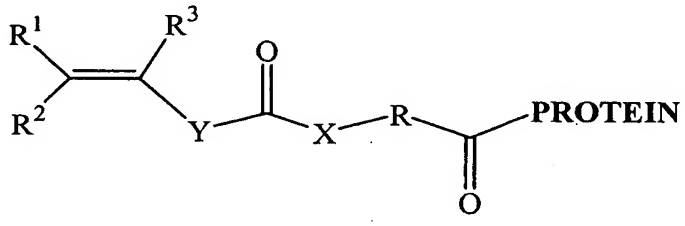
R^4 represents H, $(CH_2)_nOH$ where n = 2-6;

Z is $(CH_2)_nCH(CH_2OH)CH_2OX$ where n = 1-6; or $-(CH_2)_n-OX$ where n = 2-6;

X is a phosphodiester group binding an unsaturated moiety to 5'- and/or 3'- end of the DNA fragment,

being prepared by PCR-amplification using a synthetic primer bearing an unsaturated group at 5'- or 3'- end.

19. Modified proteins of the following structure:



V

wherein

R^1, R^2, R^3 are H, alkyl C_1-C_6, Ph, $PhCH_2$–;

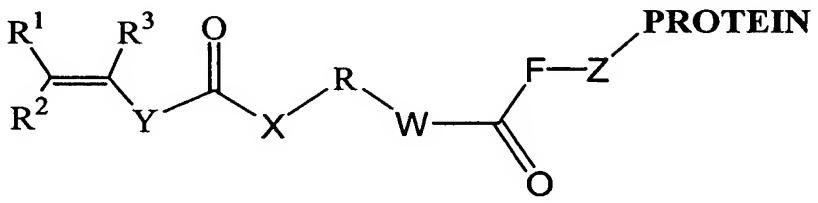
X is NH, O, CH_2, S;

Y represents $(p\text{-}C_6H_4)_n$ where n = 0-2;

R is $(CH_2)_n$, $(CH_2CH_2O)_n$, n = 1- 20,

being prepared by acylation of protein's free amino-groups with activated esters of unsaturated acids;

or



VI

wherein

R^1, R^2, R^3 are H, alkyl C_1-C_6, Ph, $PhCH_2$- ;

X is NH, O, S, CH_2 ;

Y is $(p\text{-}C_6H_4)_n$, where n = 0-2;

R is $(CH_2)_n$, $(CH_2CH_2O)_n$, n = 1-20;

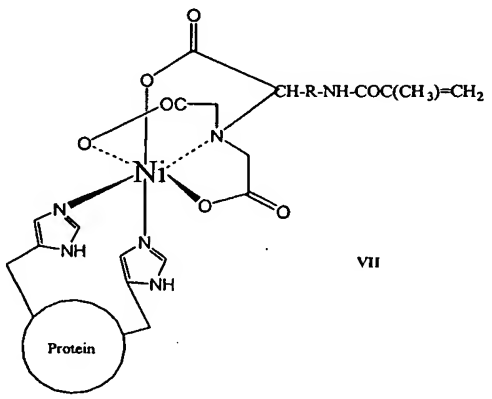
W is NH, O, CH_2;

F is $(CH_2)_n$, n=1, 2;

Z =NH, S

being prepared by alkylation of protein's amino- or sulfhydryl groups with derivatives of αβ-unsaturated and α-halocarbonyl compounds;

or

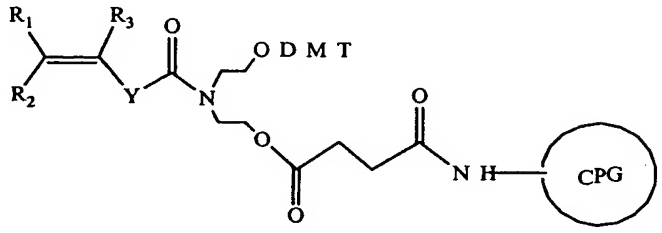


VII

wherein R represents $(CH_2)_n$, $(CH_2CH_2O)_n$, n = 1–20;

by treatment of a recombinant protein comprising an His-6 end fragment with methacrylamide derivatives of nitrilotriacetic acid in the presence of Ni(II) salts.

20. A modified porous glass (CPG) of the following structure:



wherein:

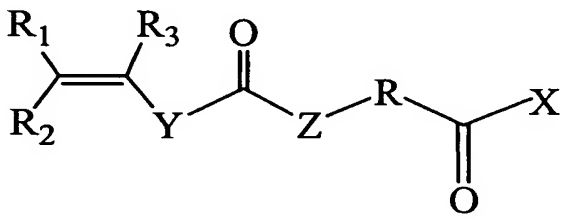
R^1, R^2 are H, alkyl C_1-C_6, Ph, $PhCH_2$-;

R^3 is alkyl C_1-C_6;

Y is $(p\text{-}C_6H_4)_n$, where n = 0-2,

as a carrier to insert the fragment of unsaturated acid at 3'-end of oligonucleotide of formula I according to claim 6 under conditions of automatic solid-phase synthesis.

21. Activated esters of the following structure:



wherein

R^1, R^2, R^3 are H, alkyl C_1-C_6, Ph, $PhCH_2$- ;

Y is $(p\text{-}C_6H_4)_n$, where n = 0-2;

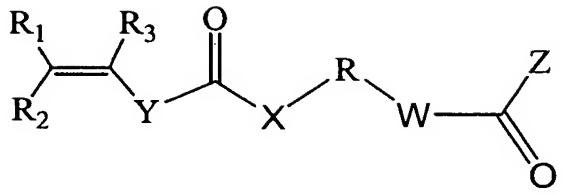
Z is NH, O, CH_2, S

R is $(CH_2)_n$, $(CH_2CH_2O)_n$, where n = 1-20;

X is succinimidoxy-, *p*-nitrophenoxy-, pentafluoro phenoxy-, or any other readily leaving acceptor group,

as a modifying agent for preparing the protein of formula V.

22. Carbonyl compounds of the following structure:



wherein

R^1, R^2, R^3 are H, alkyl C_1-C_6, Ph, $PhCH_2$- ;

Y is $(p\text{-}C_6H_4)_n$, n = 0-2;

X is NH, O, S, CH_2;

R is $(CH_2)_n$, $(CH_2CH_2O)_n$, n = 1-20;

W is NH, O, CH_2,

Z is a halomethyl, vinyl, or any other fragment comprising an active multiple bond, as a modifying agent for preparing the protein of formula **VI**.

23. Methacrylamide derivatives of nitrilotriacetic acid of general formula:

CH_2=C(Me)–CO-NH–R-CH(COOH)–N(CH_2COOH)$_2$,

wherein R is $(CH_2)_n$, $(CH_2CH_2O)_n$, n = 1-20,
as a modifying agent for preparing the protein of formula **VII**.

24. Biochip accomplished based on composition of claim 1 wherein gel layer formed on a substrate is divided by empty spaces into several cells and each cell will comprise or not comprise macromolecules immobilized, and macromolecule being immobilized in various cells will have different nature and properties.

25. Biochip according to claim 24 wherein said cells form the regular one- or two-dimensional structure (phase).

26. Biochip according to claim 24 on preparation of which an application of the polymerization mixture on substrate is preferably carried out by using an automatic device (robot) equipped with one or more micro dispensers.

27. Biochip according to claim 26 on preparation of which use is made of micro dispensers of rod type.

28. Biochip according to claim 26 on preparation of which use is made of contactless micro dispensers of jet type.

29. Biochip according to claim 26 on preparation of which use is made of several micro dispensers forming a regular structure.

30. Biochip according to claim 24 on preparation of which one or more substrates including applied droplets of polymerization mixture, during polymerization, are placed into a sealed container under oxygen free inert atmosphere with a controlled humidity.

31. Biochip according to claim 24 on preparation of which said container being filled with one of the following gases: N_2, Ar, CO_2.

32. Biochip according to claim 24 on preparation of which said gaseous medium being continuously or periodically restored in the container with substrates.

33. A method for performing the PCR over biochip according to claim 24 by using:

- an addition of amplification solution, forward (F) and reverse (R) primers of samples of nucleic acids under investigation,

and

- an incubation of biochip under conditions of a thermocycling treatment providing a realization of PCR-amplification.

34. A method for performing the PCR over biochip according to claim 24 by using:

- an isothermal incubation of biochip with hybridization solution comprising the samples of nucleic acids under investigation to perform their hybridization with primers immobilized (synthetic oligonucleotides),
- an isothermal incubation of biochip, comprising the nucleic acids being hybridized with primers immobilized, in the amplification solution containing forward (F) and reverse (R) primers,
- replacement of the amplification solution out of biochip gel elements with hydrophobic liquid (mineral oil) which completely isolates biochip cells with each other, and
- an incubation of biochip under conditions of a thermocycling treatment providing a realization of PCR-amplification.